SARS-CoV-2 BA.1 and BA.2 co-infection detected by genomic surveillance in Brazil, January 2022

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Abstract

In January 2022, the genomic surveillance of DASA laboratories identified a SARS-CoV-2 BA.1 and BA.2 co-infection in a sample from a patient resident in Brazil. The bioinformatics protocols using Dragen™ Covid Lineage App do not allow the detection of recombinants or mixed-infections. Therefore, the number of co-infections is certainly underestimated.

Full Text

On March 11th 2020, the World Health Organization (WHO) declared COVID-19 a pandemic. Since then, genomic surveillance has been performed to monitor the circulating lineages and identify the emergence of new SARS-CoV-2 variants, in particular, those of concern (VOC) [1, 2]. In November 2021, the Omicron (B.1.1.529) VOC was identified in South Africa, and it rapidly spread all over the world [2]. B.1.1.529 evolved and is now divided into sublineage BA.1 and two related sublineages BA.2 and BA.3, which share many but not all specific mutations with BA.1, having characteristic constellations of their own [3]. As some other VOCs, SARS-CoV-2 BA.1 and BA.2 share aminoacid substitutions in the spike protein (G339D, S477N, T478K and N501Y) that could enhance binding to human ACE [4]. Some RDB mutations (G496S, A67V, T95I, Del 69-70, Del 143-145 and the insertion EPE between 214-215 position) specific of SARS-CoV-2 BA.1 lineage, are predicted to substantially reduce the protection provided by SARS-CoV-2 vaccines [5]. However, humoral immunity induced by vaccines fails, at a similar extent, to protect against both BA.2 and BA.1 [6]. Moreover, the effective reproduction number of BA.1 is 1.4-fold lower than BA.2, which also reaches higher viral loads in the human nasal epithelial cells [6].

In November 30th, 2021, SARS-CoV-2 BA.1 was firstly detected in Brazil when Dasa laboratories began screening for its presence by accounting for S-gene target failures (SGTFs) during routine diagnostic PCR testing employing the Covid-19 TaqPath assay from ThermoFisher, as it was done before for the Alpha VOC, since both bear the S:del69/70 that causes the SGTF pattern. Later, in January 2022, SARS-CoV-2 BA.1 became predominant in Brazil while BA.2 was already been described in several countries and apparently spreading faster than BA.1. By January 10th, Omicron already represented 99% of the circulating SARS-COV-2 variants in Brazil. We then started to pay attention in samples without SGTF during the diagnostic PCR testing to screen for the presence of BA.2 variant.

From January 17th to 25th, we identified 47 samples with SGTP (S-Gene Target Positive) and submitted them to next generation sequencing (NGS) using the Illumina COVIDSeq Test on the NovaSeq 6000 equipment (Illumina, CA, USA) (Local Ethical approval – CAAE 45540421.0.0000.5455).

One of these samples, collected in January 19th, 2022, belonging to a 34 years old man, resident of Volta Redonda city, Rio de Janeiro state, was identified as BA.1 lineage using Dragen™ Covid Lineage App, with S:Del 69-70. Intriguingly, the TaqPath-based diagnostic PCR testing did not show SGTF (Ct values – N: 19.64; S:23.13; and ORF1ab: 19.35), so we decided to investigate this isolate in more detail.

A maximum likelihood phylogenetic tree including this and other Brazilian sequences sampled in the same period was built using IQTREE2 (iqtree.org). The dataset also included complete genomes of Omicron BA.1, BA.2 and BA.3; Delta and the recombinant XD as references. As expected for recombinant or co- infection resulting consensus genomes, the sample clustered with a long branch and low bootstrap support within BA.1 clade. (Figure A). This sample was deposited in the GISAID database under EPI_ISL_11271349 ID and presented 99.59% of non-N bases with a mean coverage of 2,833. NextClade pointed several labeled private mutations associated to the 21L clade, although the Pango designation assigned it as BA.1 lineage. We analyzed the mutation profile of the reads obtained in the NGS and observed the presence of SARS-Cov-2 BA.1 characteristic mutations in the spike gene (A67V, T95I, Y145D, G496S, T547K, N856K, L981F, del 69-70 and del 142-144), as well as SARS-CoV-2 BA.2 specific mutations (T19I, V213G, S371F, T376A, D405N and R408S) in different proportions, strongly indicating that this sample was not a recombinant BA.1/BA.2, but instead a co-infection of both lineages (Table).

Figure B illustrates the mix present in the regions 21765-21770 and 21633-21641 where SARS-CoV-2 BA.1 and BA.2 are expected to have deletions, respectively. To confirm the observation, we performed an RT-PCR using specific probes to the del 69-70 and wild type detection and both variants were amplified. Unfortunately, most samples sent to us to SARS-COV-2 surveillance do not have associated clinical data such as vaccination history.

Previous co-infections with SARS-CoV-2 sublineages have been reported [7–10]. However, this is the first report of BA.1 and BA.2 sublineages co-infection that we have noticed. The SARS-Cov-2 variants co-infection should be monitored since it is a *sine qua non* condition to the emergency of recombinant viruses. Unfortunately, SARS-CoV-2 genomic surveillance is not ideal in many countries and rare events like these are likely unnoticed. Also, the standard bioinformatics protocols using Dragen™ Covid Lineage App and the absence of a detailed phylogenetic or visual inspection in all genomes generated do not allow the clear detection of putative recombinants (as happened to the “Deltacron”) or mixed-infections. Therefore, the number of co-infections with different SARS-CoV-2 sublineages is certainly underestimated.

Declarations

Funding

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Table

Table: Percentage of bases in key positions of the spike gene able to discriminate between SARS-CoV-2 BA.1 and SARS CoV-2 BA.2

SARS-CoV-2 lineage	Mutation	Nucleotide Position	Nucleotide (%)
BA.2	T19I	21,618	C (69), T (28)
BA.1	A67V	21,762	T (56), C (43)
BA.1	T95I	21,846	T (66), C (33)
BA.2	V213G	22,200	G (93), T (7)
BA.2	T376A	22,688	G (95), A (4)
BA.2	D405N	22,775	A (96), G (3)
BA.2	R408S	22,786	C (96), A (4)
BA.1	G496S	23,048	A (71), G (26)
BA.1	T547K	23,202	A (58), C (41)
BA.1	N856K	24,130	A (64), C (35)
BA.1	L981F	24,503	T (68), C (32)

Figures

Figure 1

(A) Maximum likelihood phylogenetic tree including the study sequence and other Brazilian sequences sampled in the same period build using IQTREE2. (B) Assembly of the reads of the study sequence showing lower coverage in regions 21765-21770del and 21633-21641del, where SARS-CoV-2 BA.1 and BA.2 were expected to have deletions, respectively.